In the name of God

Cell Penetrating Peptid(CPP)

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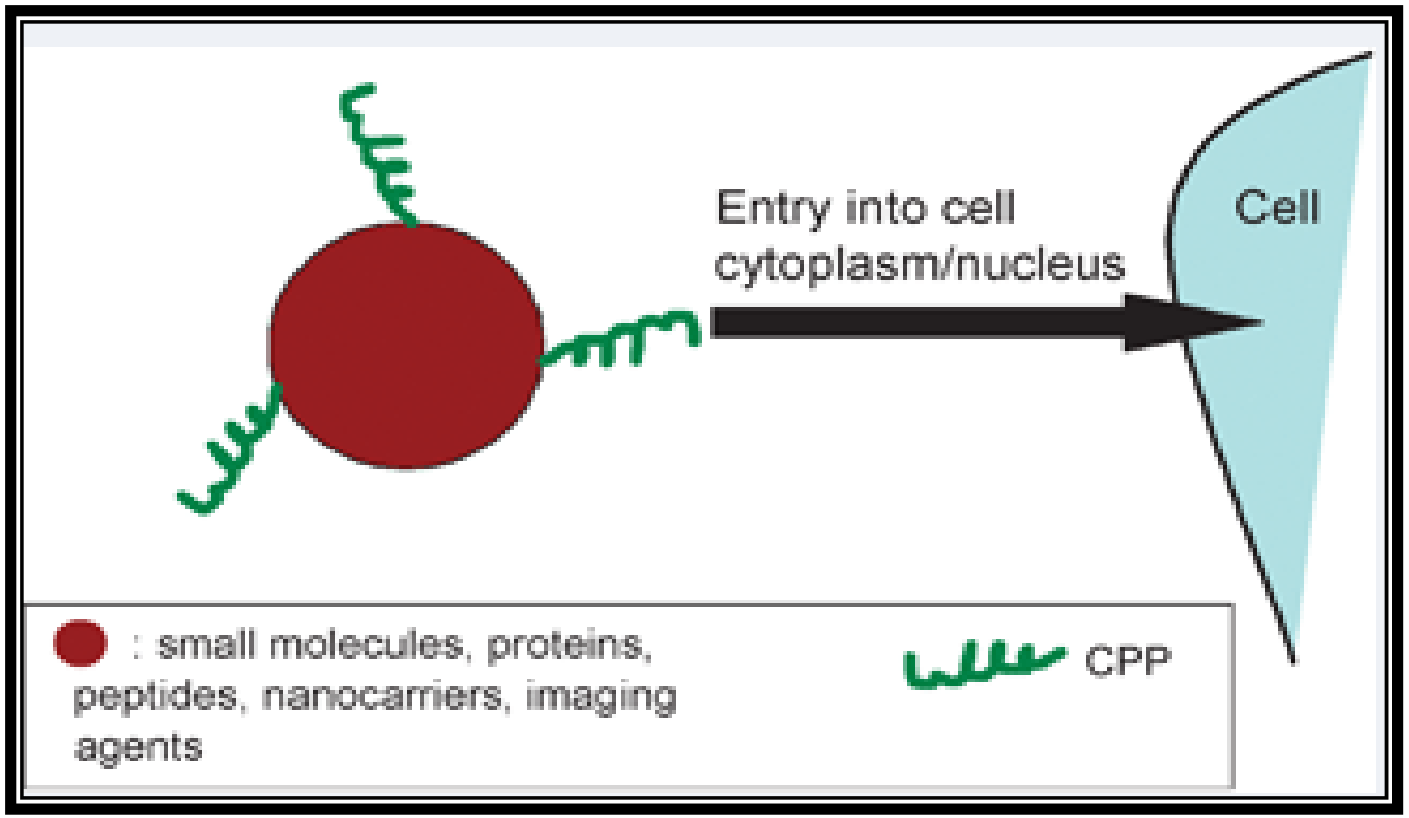
CONTENTS

INTRODUCTION

Translocate across plasma membranes in a nonreceptor-mediated fashion(b1)

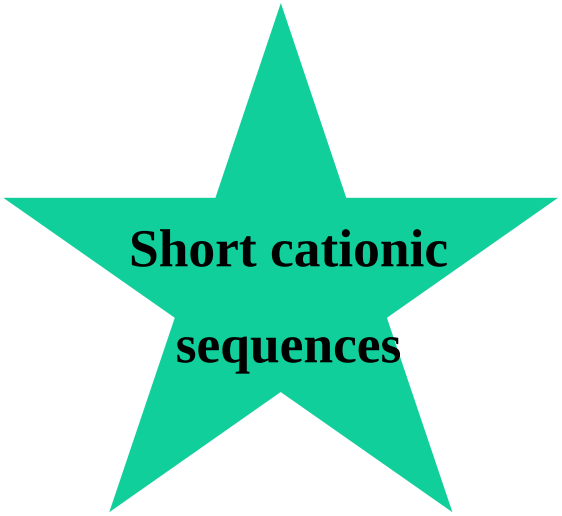


CPP:Intracellular delivery vehicle

Cargo: Drug,protein,SiRNA,PNA,plasmid,etc

CPP (5-30 aa)

HISTORY



- In 1988
  - HIV-coded Tat regulatory protein
  - Taken up by cells in culture media
- In 1991
  - pAntp(43–58) (later called penetratin)
  - The homeodomain of Antennapedia (*Drosophila homeoprotein*)
  - Internalize via receptor-independent manner
  - Studied by site-directed mutagenesis;
  - 43 to 58 amino acids necessary for translocation

CPPs

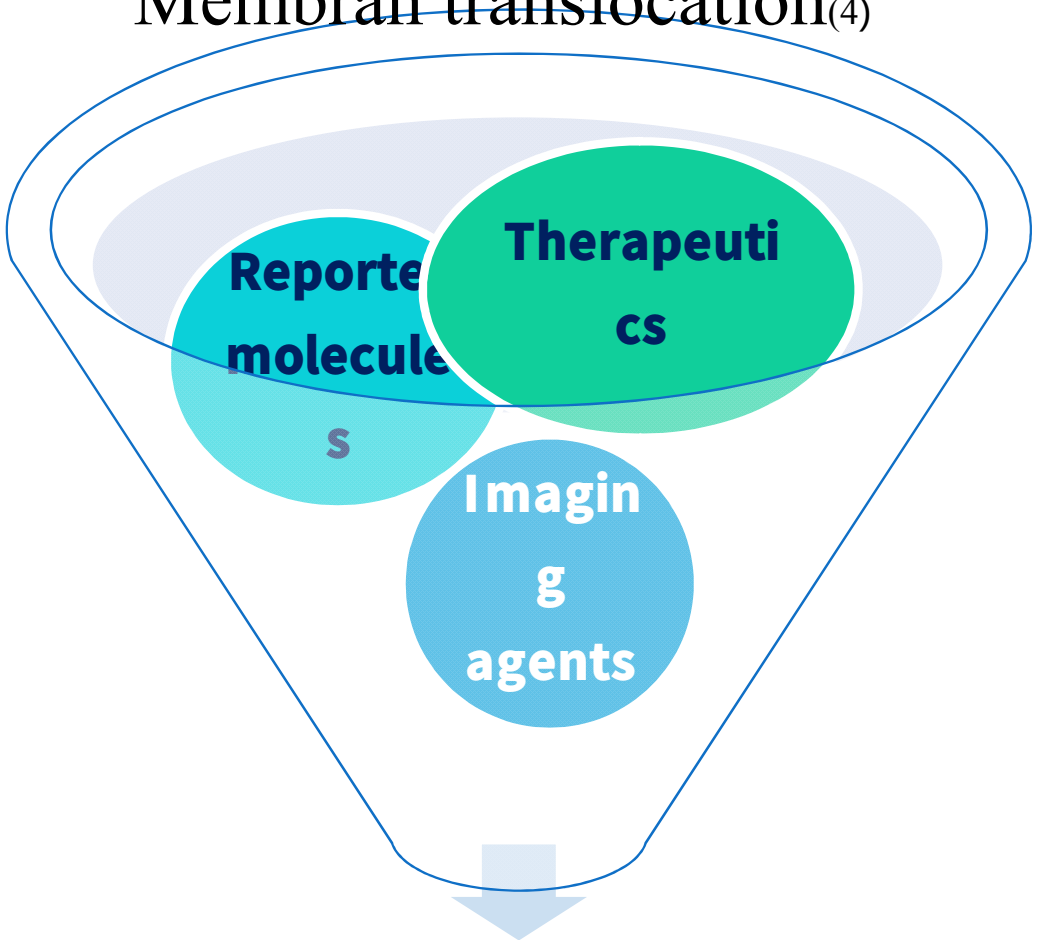
- Also called:
  - PTDs(Protein Transduction Domains)
  - AID(arginine-rich intracellular delivery)

- Be useful for the treatment of

  inflammatory bowel disease, diabetes and muscular dystrophy

CPP'S MISSION

Membran translocation(4)



Cytosol/Nucleus

Drug carrier properteis

- Be biodegradable and biocompatible
- Lack intrinsic toxicity and antigenicity,
- Show no accumulation in the body,
- Maintain the delivered drug activity until it reaches the site of action(6)

ADVANTAGES OF CPPS

- **Have remarkably low toxicity**
- **Have an extensive range of possible cargo types**
- **Unlike viral vectors, do not integrate the genetic material they deliver**

ADVANTAGES OF CPPS

- **Successful delivery to numerous cell types (unlike liposomes)**
- **Resist degredation (after internalization)**
- **adding homing sequences to the CPP**(5)

Classification(5)

Protein derived	chimeric	Designed/synthetic
Penetratin(pAnt) (Drosophila Antennapedia)	MPG(HIV fused with T-Ag of SV40 virus)	MAP(amphipathic model peptide)
Tat (48-60) (HIV-1)	Pep-1(SV40)	CADY(derived from PPTG1 peptide)
pVEC(VE-cadherin)	Transportan/TP10 (Galanin and mastoparan)	POD(peptide for ocular delivery)
VP22 peptide (Herpes simplex virus)		

TAT

- **Tat uptake is :**
  - **Time-independent**
  - **Concentration dependant**
  - **Partially inhibited by loweing temprature**
- **Tat** deliver beta-galactosidase (b-Gal) to all tissues
- Peptides fused to TAT enter cells within seconds*

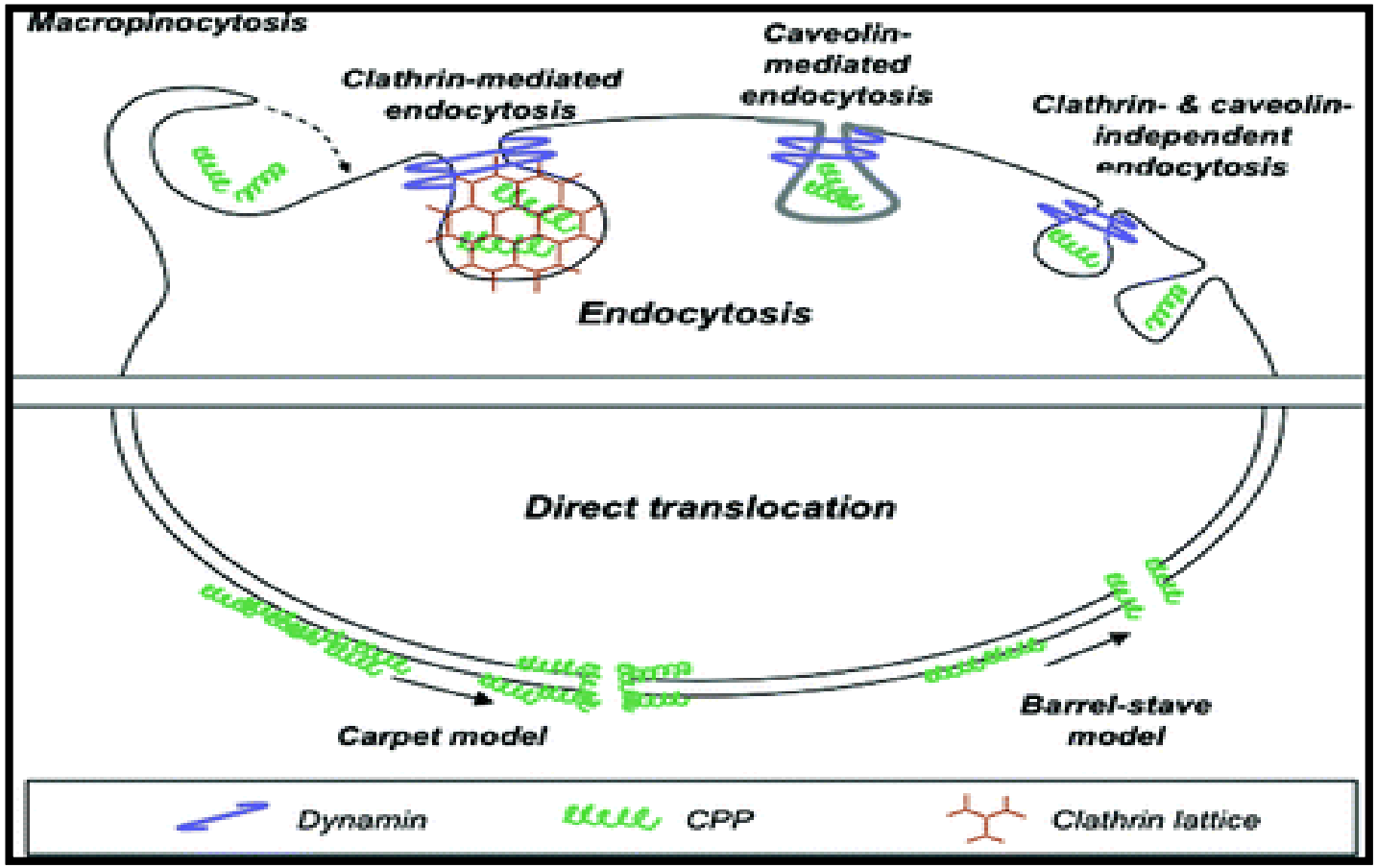
TAT

- In the construction of **nanocarriers**

delivery the drugs to tumors

- Enable delivery of molecules useful in treatment of **allergies** and production of **vaccines**

UPTAKE MECHANISM



Mechanisms of uptake across the plasma membrane(3)

Each CPP expresses its own preferred mechanism of uptake(1)

MULITIPLE MODES OF CELLULAR ENTRY (4)

- **Energy-dependent endocytosis**
  - ✓ **Macropynocytosis**
  - ✓ **Clatrin-mediated endocytosis**
  - ✓ **Caveolin/lipid raft mediated endocytosis**
- **Energy-independent direct translocation**

Factors that affect the uptake mechanism(4)

- Various properties of peptides, such as:
  - molecule length
  - charge delocalization

- the properties of the associated cargo, such as :
  - size
  - Charge

APPLICATIONS

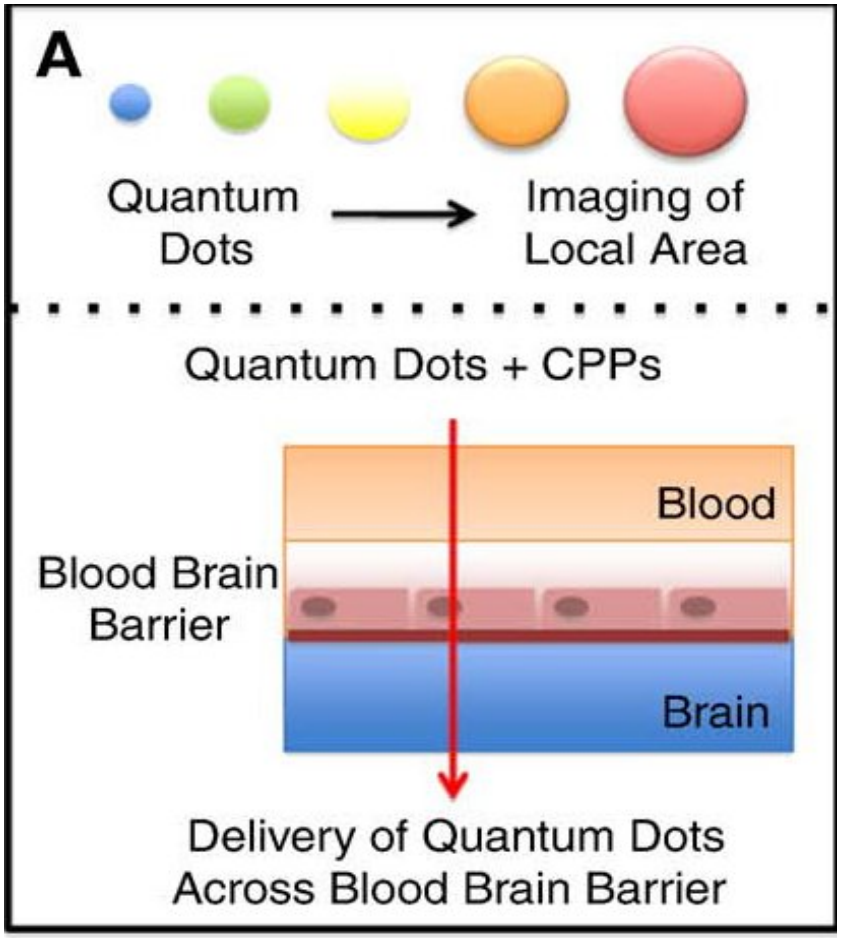
Fields of applications(4)

- In imaging and biosensing applications
- In bioactive cargo delivery
- In cancer therapy
- Covering cellular territory

Imaging and Biosensing(4)

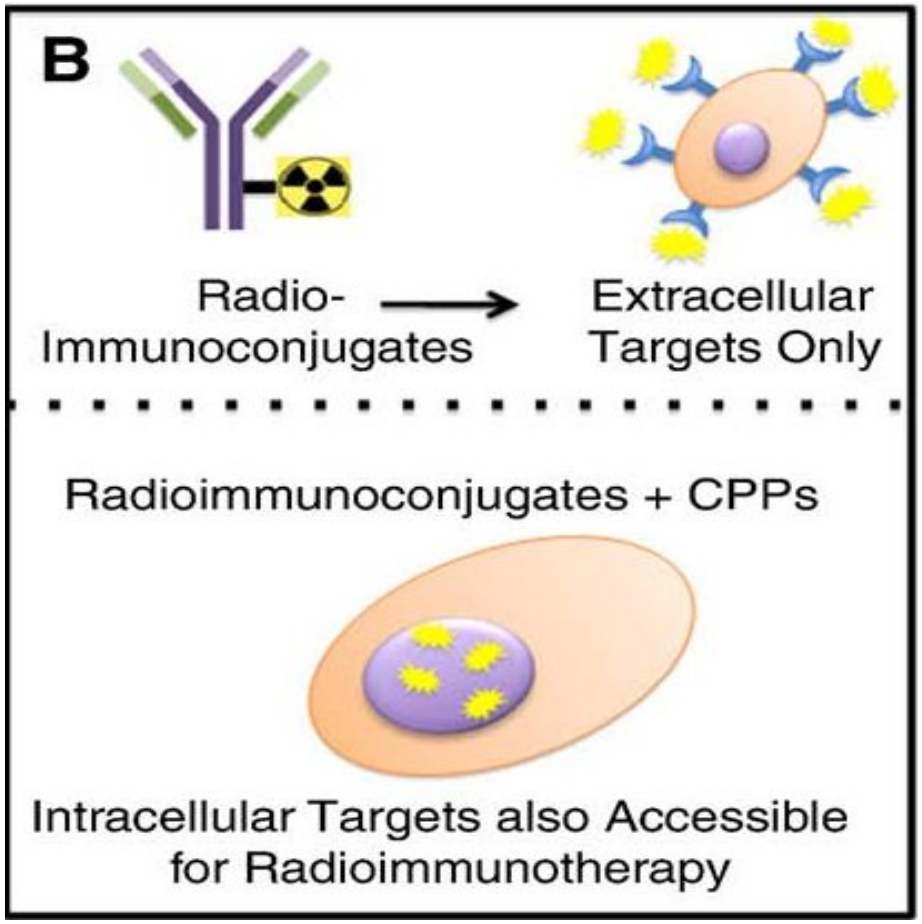
- ✓ Deliver quantum dots across BBB
- ✓ Deliver antiobiotics
- ✓ Visualize viral infections
- ✓ Deliver intracellular biosensors

DELIVERING QUANTUM DOTS

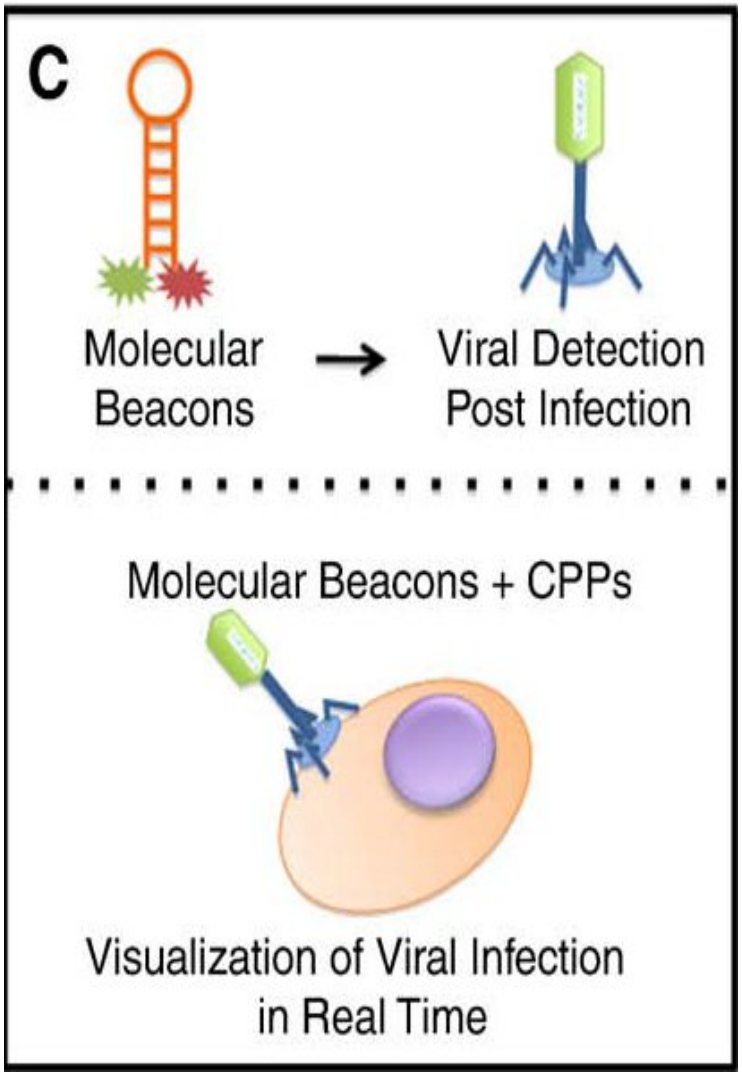


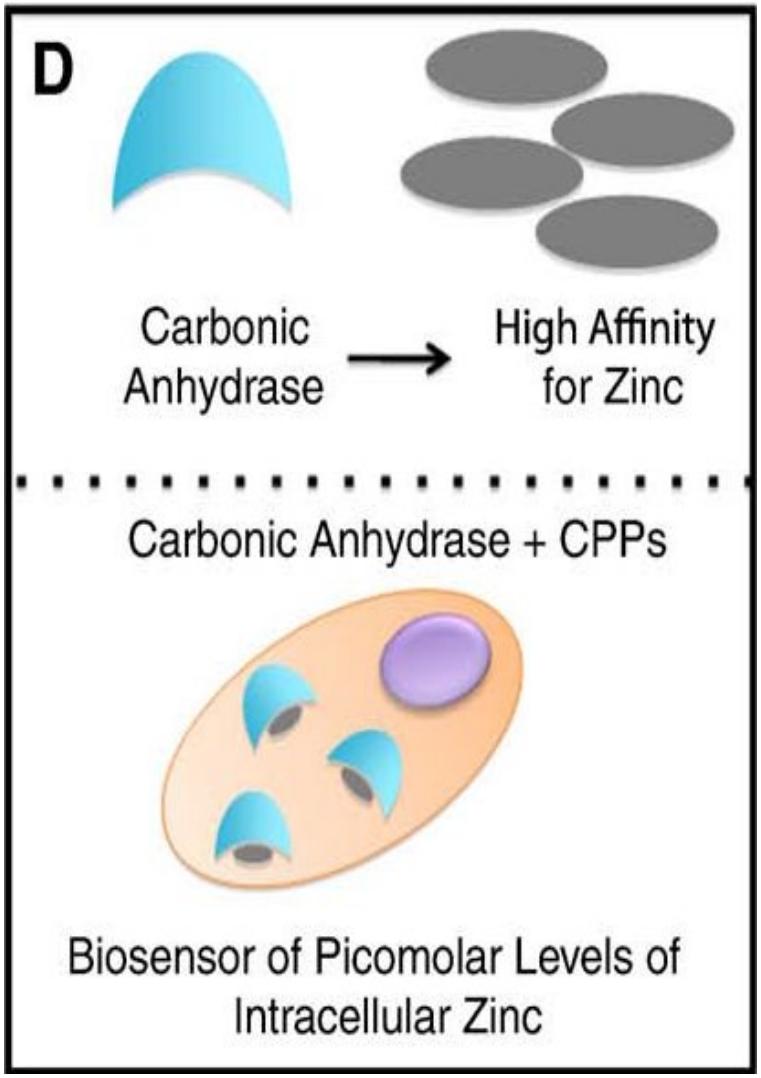
Quantum dots conjugated to CPPs are able to cross the highly impermeable blood-brain barrier(4)

DELIVERING ANTIOBIOTICS



Radioimmunoconjugates can
access intracellular molecules upon conjugation to CPPs(4)

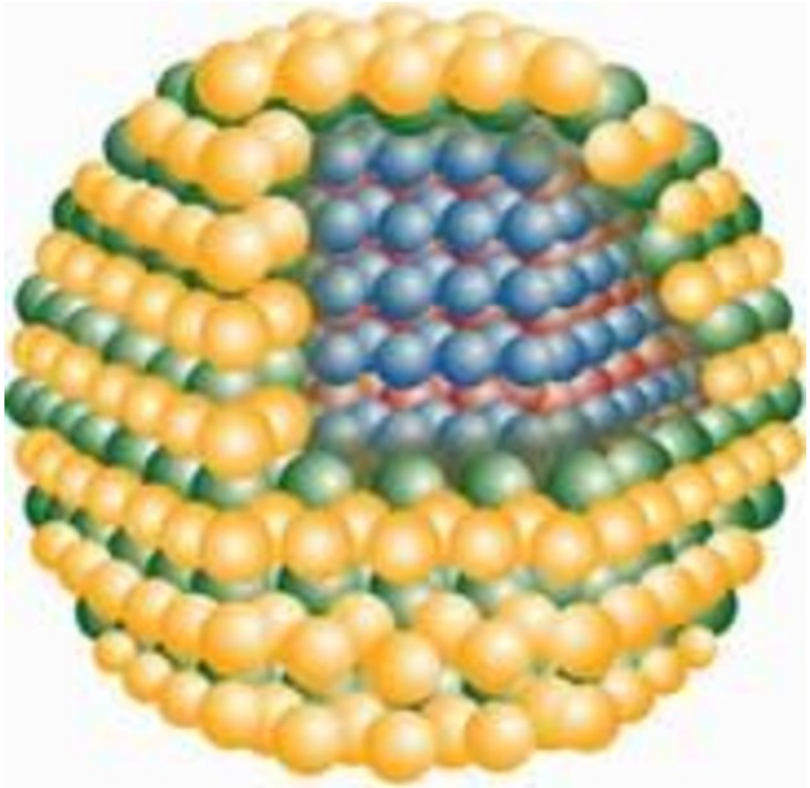




Molecular beacons attached to CPPs can be harnessed to visualize viral infection in real time(4)

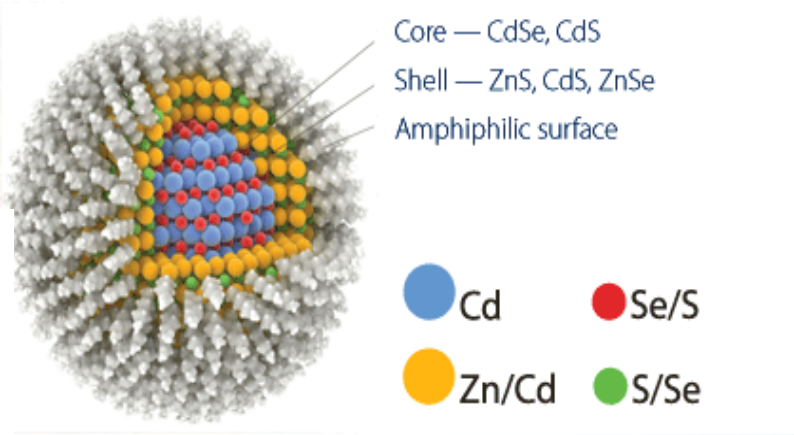
Carbonic anhydrase, has been conjugated to CPPs to yield a highly sensitive biosensor for measuring intracellular zinc levels(4)

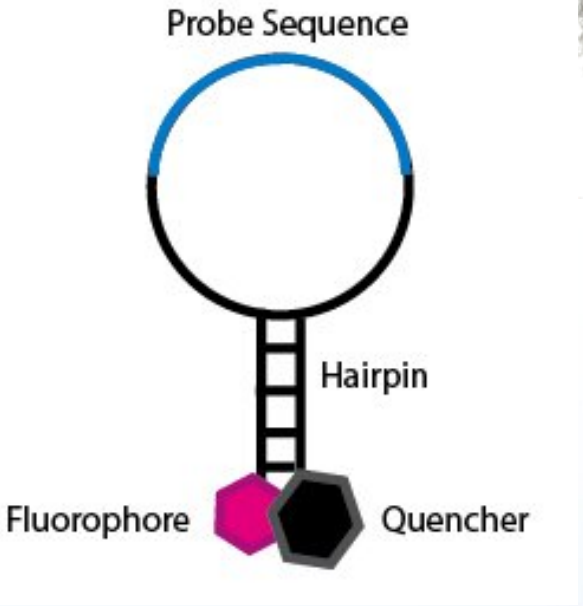
What is a quantum dot?

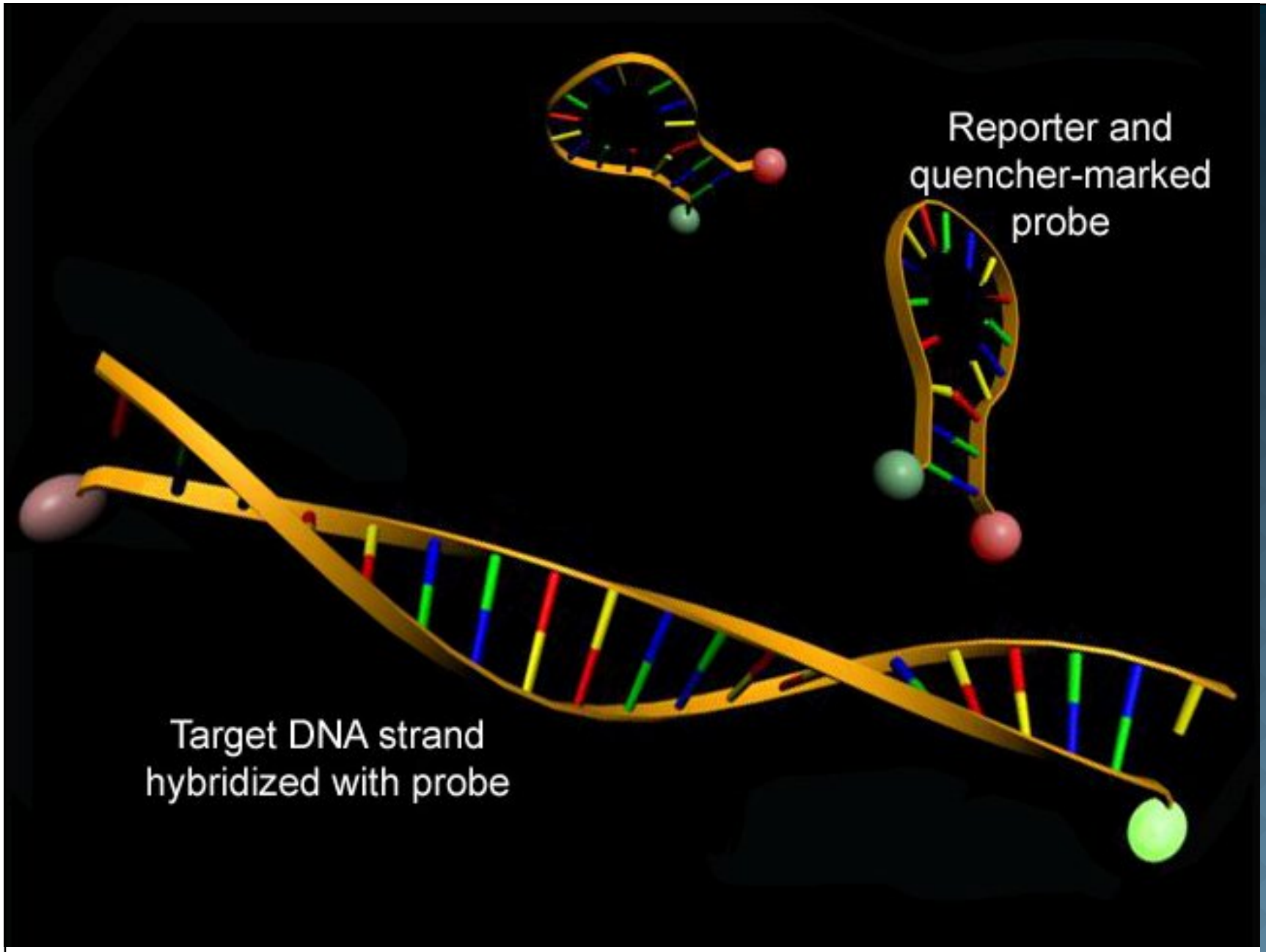


Core-Shell EviDot

- Nanocrystals
- 2-10 nm diameter
- semiconductors


Core — CdSe, CdS
Shell — ZnS, CdS, ZnSe
Amphiphilic surface
Cd
Se/S
Zn/Cd
S/Se


Probe Sequence
Hairpin
Fluorophore
Quencher

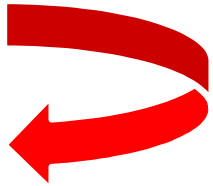


Structure of molecular beacons in their native conformations (top) or hybridized with a DNA strand (bottom)

HR9 PEPTIDES

(HISTIDINE- ARGININE RICH)

- Delivery of QDs congugated with CPPs
- Via endocytic pathways



- combined with HR9
  - _ Stably but noncovalently conjugated
  - _ Entered in 4min
  - _ Penetrates cell membrane directly*
  - _ Stay in cytosol without any organelle capture

HR9

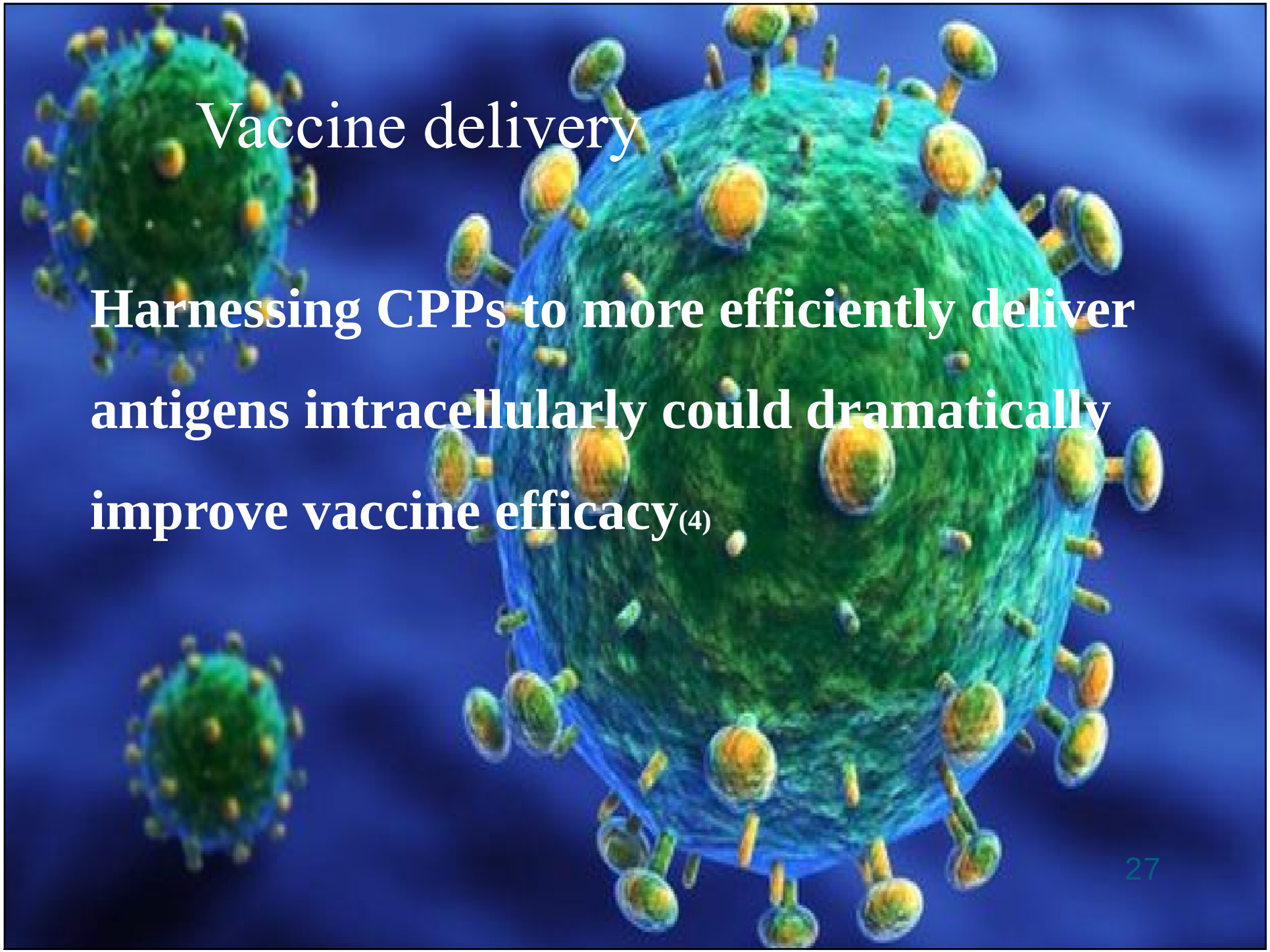
- Dimethyl sulphoxide, ethanol and oleic acid

enhanced intracellular delivery of HR9-QDs

(by promoting the direct membrane translocation)

- HR9 → no cytotoxicity

  → an efficient carrier to deliver drugs without interfering with their therapeutic activity

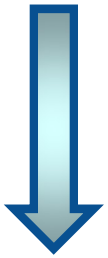


Vaccine delivery

Harnessing CPPs to more efficiently deliver antigens intracellularly could dramatically improve vaccine efficacy(4)

CANCER THERAPY(4)

- Increase appoptosis on drug resistant cancer cell lines
- Increase the efficacy of cancer drugs used in chemotherapy
- Combining CPPs with tumour homing domains

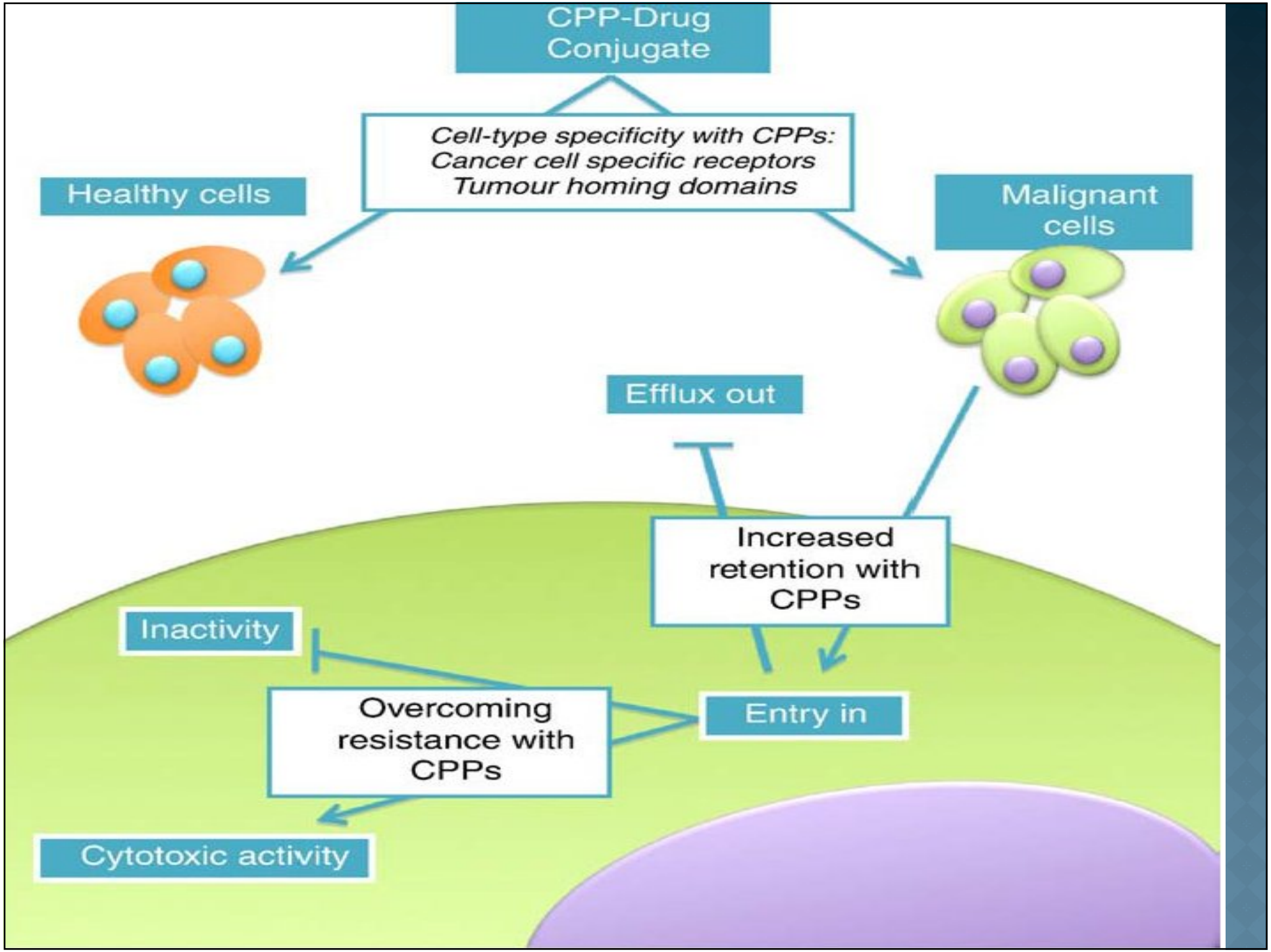


Selectively delivery to cancer cells

Conjugation of CPPs to drug molecules allows For:

- **Increased retention in tumours** (by decreasing efflux out of the cell)

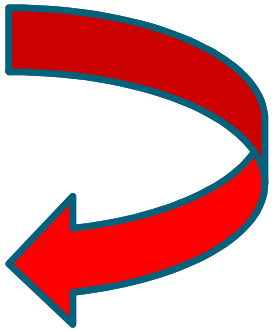
- **Overcome drug resistance**(by increased intracellular drug concentrations in tumour cells)(4)


CPP-Drug Conjugate
Cell-type specificity with CPPs:
Cancer cell specific receptors
Tumour homing domains
Healthy cells
Malignant cells
Efflux out
Increased retention with CPPs
Inactivity
Overcoming resistance with CPPs
Entry in
Cytotoxic activity

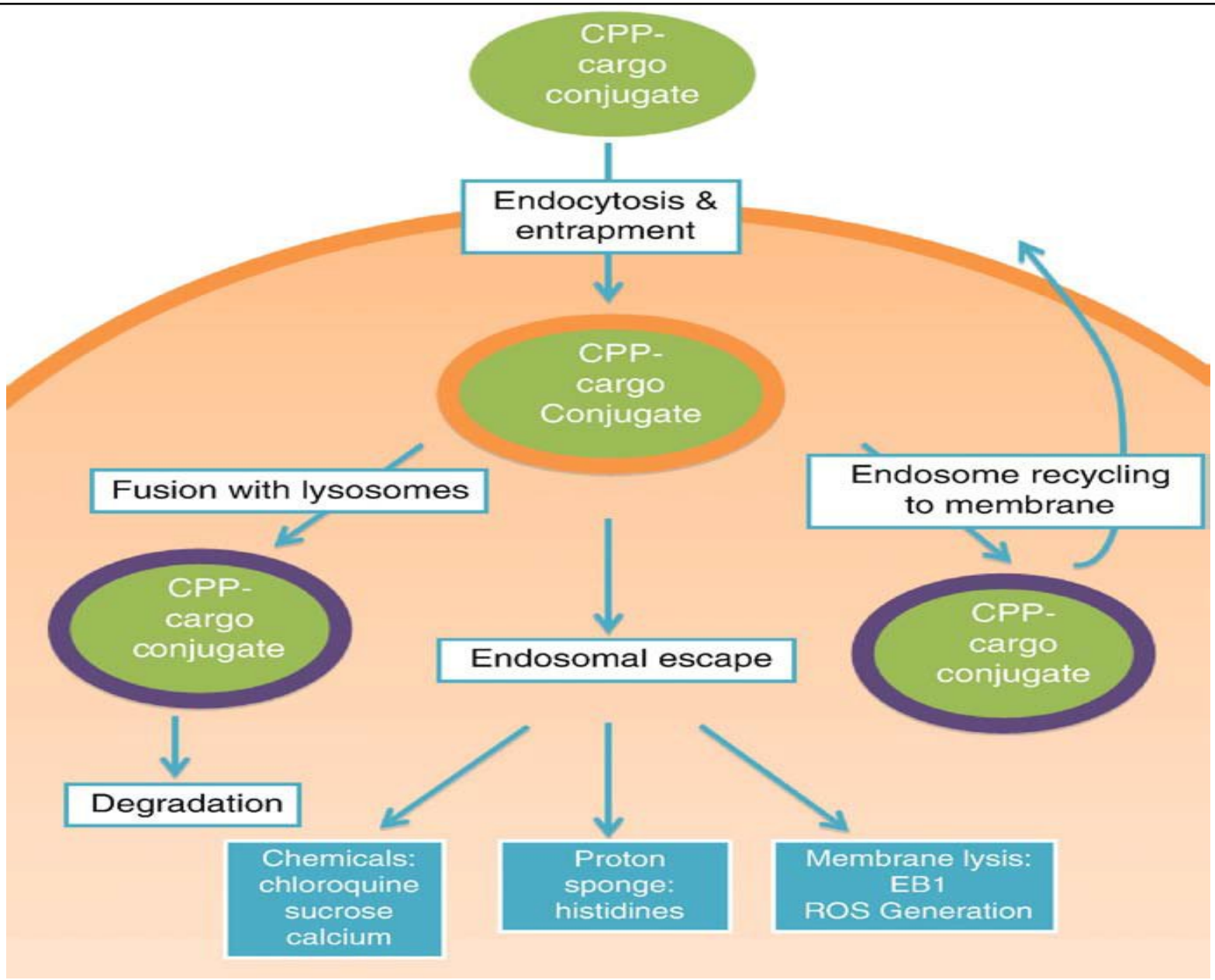
NOVEL CPPS

Covering cellular territory

- They often remain sequestered in endosomes
- CPPs being modified to improve endosomal scape



- ✓ To deliver cargo to subcellular organelles
- ✓ To deliver specially anionic cargo



Improving endosomal escape to achieve increased biological activity(4)

NON-COVALENT INTERACTIONS

- **why this is advantegous?**

A. **Simplifies conjugation protocols**

B. **Delivering different cargo simply**(by mixing the two Compounds)

C. **Dicrease the likelihood of interfering with the bioactively of the payload**

NON COVALENT CPP

- One such CPP is:
- **Rath peptide**,derived from the avian infectious virus
  - It adopts a dominant β-structure, unusual for CPPs
  - Delivery of both proteins and nucleic acids
  - The delivery is rapid (30 min for an oligonucleotide and 1 h for an antibody)

NON COVALENT CPP

- Temperature independent,
- A nonendocytic mechanism of uptake
- It avoids endosomal entrapment

- CADY is another this kind of peptide

ORAGANELL SPECIFIC CPPS(4)

Example:

- **MPPS** (mitochondria-penetrating peptides)

- ✓ By making a balance between cationic character and hydrophobicity

- ✓ gain mitochondrial access efficiently

- ✓ Study the cellular responses to ROS

Histatin 5

- A antimicrobial peptide
- Found in human salvia
- It’s target fungal and protozoan mitochondria
- Accumulate within the mitochondria and inhibit F1F0-ATPase
- Causes collaps of the parasite(4)

REFRENCES

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THANKS FOR YOUR
ATTENTION